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Heterocyclic Insecticides Acting at the GABA-Gated Chloride Channel: 5-Alkyl-2-arylpyrimidines and -1,3-thiazines

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Abstract: 5-*tert*-Butyl-2-(4-ethynylphenyl)pyrimidine and the corresponding 2,5-disubstituted-4*H*-1,3-thiazine block the GABA-gated chloride channel at *c.*20 and *c.*200 nM, respectively, measured as 50% inhibition of the binding of 1-(4-ethynylphenyl)-4-[^{3}H]propyl-2,6,7-trioxabicyclo[2.2.2]octane (4′-ethynyl-4-*n*-[^{3}H]propylbicycloorthobenzoate; [^{3}H]EBOB) in house fly and mouse brain membranes, and they are also toxic to topically-treated flies with LD_{50} values of 6–27 $\mu g\ g^{-1}$ alone and 2–6 $\mu g\ g^{-1}$ with piperonyl butoxide (PB) as synergist. In the pyrimidine series, the general pattern of effectiveness of substituents in the 5-position is *tert*-butyl > isopropyl ≈ cyclohexyl ≈ cyclopropyl > methyl, phenyl and 3- and 4-fluorophenyl, and in the 2-position is 4-ethynylphenyl ≫ 4-bromophenyl. These planar pyrimidines and nearly-planar 4*H*-1,3-thiazines with 2-ethynylphenyl or 2-bromophenyl and 5-*tert*-butyl or 5-isopropyl substituents are more effective than the corresponding 6*H*-1,3-thiazine, 6-oxo-1,3-thiazines and 4,6-dioxo-1,3-thiazine examined, but they are less active than the analogous conformationally flexible *trans*-1,3-dioxanes and -1,3-dithianes. The heterocyclic moiety confers a region of high electron density and positions the 2- and 5-substituents in a linear or parallel relationship for optimal affinity at the receptor. Two observations indicate that the new pyrimidines and thiazines probably act as chloride channel blockers. First, the poisoning signs are identical to those of EBOB in both mice and house flies. Second, each of the pyrimidines, thiazines and dioxanes falls on the same correlation line for inhibition of [^{3}H]EBOB binding and toxicity to house flies (with PB) as that obtained earlier for EBOB analogs, dithianes and polychlorocycloalkanes, suggesting that they all act at the same or closely coupled binding sites in the GABA-gated chloride channel.

Key words: γ-aminobutyric acid; chloride channel; dioxanes; EBOB; ethynylpropylbicycloorthobenzoate; heterocyclic insecticides; house fly; pyrimidines; thiazines

1 INTRODUCTION

Suitably substituted 4-alkyl-1-aryl-2,6,7-trioxabicyclo-[2.2.2]octanes and 5-alkyl-2-aryl-1,3-dithianes are among the most potent blockers of the 4-aminobutyric acid (GABA)-gated chloride channel[1,2] and they also have high insecticidal activity.[3,4] The 1,3-dioxanes appear to be less active than the corresponding 1,3-dithianes.[4,5] Other heterocyclic systems that confer high activity are 1,3-oxathiane and its sulfoxides and sulfone[5–7] and the sulfoxides and sulfones of the 1,3-dithiane.[2,7] The heterocyclic component of these compounds performs two functions in providing optimal fit in the receptor: it serves as a spacer unit properly positioning the R and R′ substituents in a linear or parallel relationship (Fig. 1);[4] it confers suitable steric features and electrostatic fields.[8]

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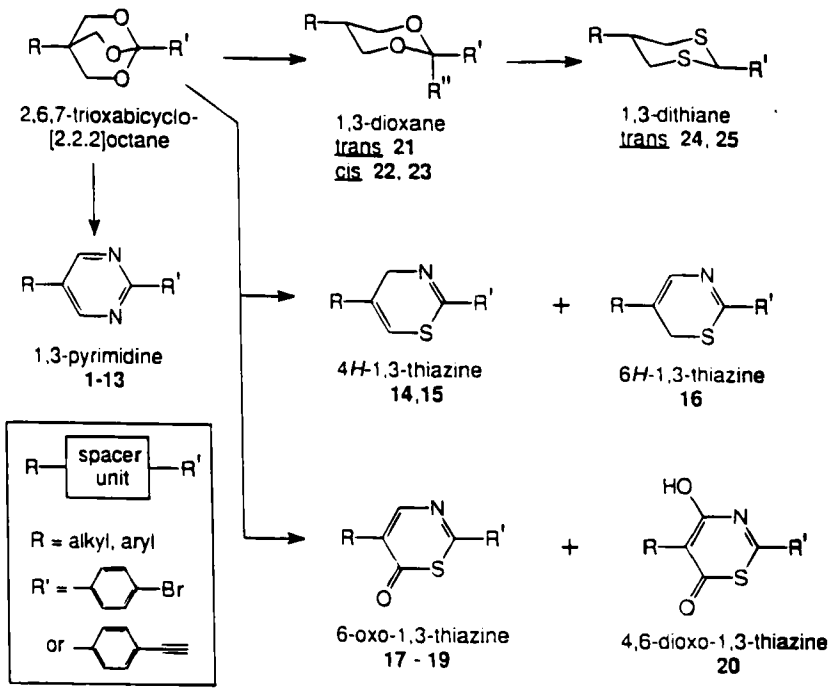


Fig. 1. Relationship between 5-alkyl-2-arylpyrimidines and -thiazines and earlier heterocyclic insecticides. In the *trans*-dioxane R′ = Ar and R″ = H and in the *cis*-dioxanes R′ = CH_3 and R″ = Ar.

This investigation uses structure—activity criteria to probe the limiting requirements of the central heterocyclic ring (Fig. 1). Planar heterocyclic systems such as pyrimidines or the 1,3-thiazines might still maintain the 2- and 5-substituents in the same linear relationship as that of the 1- and 4-substituents of the trioxabicyclooctane. In these cases the nitrogen and sulfur atoms supply the suitable electrostatic field which mimics that of the oxygen atoms of the trioxabicyclooctanes. The preferred substituents selected for examination in the pyrimidines and thiazines are 4-bromophenyl or 4-ethynylphenyl at the 2-position and *tert*-butyl or isopropyl at the 5-position, since they are expected to confer high potency, based on analogy with findings on trioxabicyclooctanes[1,3] and dithianes.[2,4] The first goal is to determine the effect on biological activity of introducing nitrogen, using a planar spacer unit such as the pyrimidine and in the slightly out-of-plane 1,3-thiazine and related compounds. The second aim is to test the hypothesis that each type of compound acts in the same way at the GABA-gated chloride channel, evaluated by correlating the potency for blocking 1-(4-ethynylphenyl)-4-[^{3}H]propyl-2,6,7-trioxabicyclo[2.2.2]octane (4′-ethynyl-4-*n*-[^{3}H]propylbicycloorthobenzoate; [^{3}H]EBOB) binding to house fly head membranes with toxicity to house flies.[9]

2 CHEMISTRY

2.1 Spectroscopy

[^{1}H] and [^{13}C] nuclear magnetic resonance (NMR) spectra (acquired at 300 and 75 MHz, respectively), for confirmation of structures and assessment of purities, were determined for solutions in deuterochloroform using a Bruker AM-300 spectrometer with stereochemical assignments for compounds **22** and **23** by nuclear Overhauser effect differential spectroscopy. Mass spectra were determined by electron impact at 70 eV using a Hewlett-Packard 5985 system.

2.2 Pyrimidines (1–13) (Fig. 2) (Table 1)

The 2-(4-bromophenyl)pyrimidines (**6–13**) were prepared by condensation of the substituted enamines (**A**) with 4-bromophenylamidine (**B**)[10] in basic solution.[11] All of the enamines are reported in the literature, i.e. methyl,[12] branched-alkyl,[13] cycloalkyl,[14] and aryl.[15] The ethynyl substituent was introduced with trimethylsilylacetylene using palladium coupling conditions followed by treatment with tetrabutylammonium fluoride.[16]

2.2.1 2-(4-Bromophenyl)-5-tert-butylpyrimidine (6) and 5-tert-butyl-2-(4-ethynylphenyl)-pyrimidine (1)

A solution of **A** (R = *tert*-butyl) (0·3 g) and **B** (0·5 g) in dry ethanol (10 ml) was stirred under nitrogen while sodium methoxide (2 M; 1·8 ml) was added. The mixture was heated at reflux for 10 h, cooled, and treated with 10% aqueous potassium hydroxide (100 g litre^{-1}; 5·5 ml). After stirring the mixture overnight at room temperature, the solvent was evaporated and water and diethyl ether were added. After separation, the ethereal solution was dried (sodium sulfate) and evaporated. Flash column chromatography on

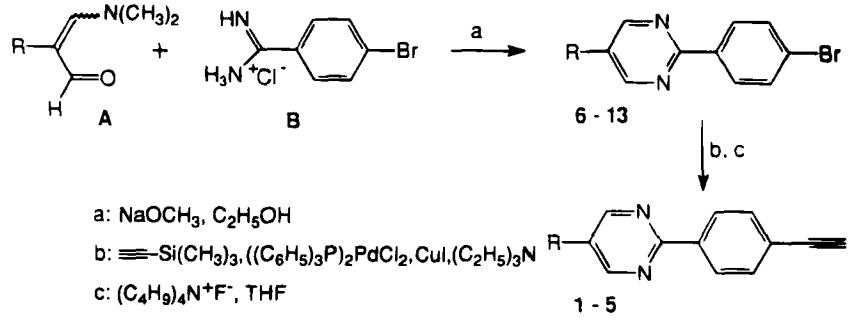


Fig. 2. Synthesis of 5-alkyl- or 5-aryl-2-arylpyrimidines. In A $N(CH_3)_2$ is replaced by OH when R is alkyl.

silica, eluting with ethyl acetate + hexane (1 + 1 by volume) yielded **6** (200 mg). [^{13}C]NMR: δ 161·0 (C-2), 154·9 (C-4), 141·1 (ArC-1), 136·6 (C-5), 131·9 (ArC-3), 129·6 (ArC-2), 125·2 (ArC-4), 32.7 (CH_3), 30·8 ($C(CH_3)_3$).

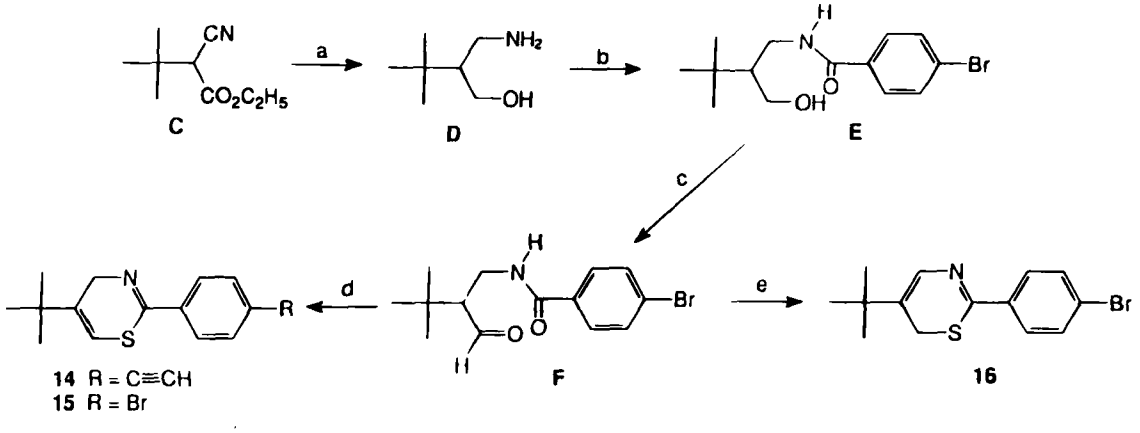
A portion of **6** (30 mg) was converted to **1** by dissolving in dry triethylamine (10 ml), then stirring under nitrogen while treating with trimethylsilylactetylene (1 ml). *bis*-Triphenylphosphinepalladium (II) chloride (100 mg) and copper (I) iodide (10 mg) were added and the mixture heated at reflux overnight. After evaporation, water and diethyl ether were added and the mixture separated. The organic solution was dried (sodium sulfate) and evaporated. The residue was dissolved in dry tetrahydrofuran (THF; 8 ml), cooled to 0°C, and stirred under nitrogen while being treated with tetrabutylammonium fluoride (1 M in THF, 0·5 ml) for 2 h, followed by evaporation and processing as above. The residue was chromatographed on silica preparative TLC with hexane + ethyl acetate (20 + 1 by volume) to obtain **1** (20 mg) as a colorless solid. [^{13}C]NMR: δ 161·0 (C-2), 154·0 (C-4), 141·0 (ArC-1), 137·8 (C-5), 132·3 (ArC-3), 127·7 (ArC-2), 123·9 (ArC-4), 83·5 (C≡CH), 77·0 (C≡CH), 32·5 (CH_3), 30·6 ($C(CH_3)_3$).

2.3 4*H*- and 6*H*-1,3-Thiazines (14–16) (Fig. 3) (Table 1)

Lithium aluminium hydride (LAH) reduction of ethyl 2-cyano-3,3-dimethylbutyrate (**C**) gave the amino alcohol (**D**). Treatment of **D** with 4-bromobenzoyl chloride gave amide **E** which was subjected to Swern oxidation to obtain aldehyde **F** followed by reaction with Lawesson's Reagent to yield the 4*H*-1,3-thiazine (**15**). The isomeric 6*H*-1,3-thiazine (**16**) was prepared from **F** using P_2S_5 followed by prolonged stirring with aqueous sodium hydrogen carbonate. The acetylene function was introduced into **15** to obtain **14** as described for the pyrimidines.

2.3.1 3,3-Dimethyl-2-hydroxymethylbutyl-1-amine (D)

A solution of **C** (5·5 g) in dry diethyl ether (70 ml) was added under nitrogen to a stirred suspension of LAH (1·4 g) in diethyl ether (10 ml). The mixture was heated at reflux for 2 h, cooled, and treated with aqueous sodium hydroxide (200 g litre^{-1}). The ethereal solution was decanted and the residue washed with diethyl ether. The combined organic solution was dried (sodium sulfate) and evaporated to give a mobile liquid (3·6 g).



a: LAH, $(C_2H_5)_2O$; b: Br-⟨⟩-COCl, NaOH, H_2O, $(C_2H_5)_2O$; c: $(COCl)_2$, DMSO, CH_2Cl_2, $(C_2H_5)_3N$;
d: Lawesson's Reagent, xylene; e: P_2S_5, xylene, $NaHCO_3$, H_2O

Fig. 3. Synthesis of 2-aryl-5-*tert*-butyl-6*H*- or 4*H*-1,3-thiazines.

TABLE 1
Structures and Characterization for Aryl Heterocyclic Compounds[a]

No.	R-heterocycle-R' R at C-5	R' at C-2	m.p. (°C)	MS $[M]^+$	$[^1H]NMR$ (deuterochloroform) (ppm)
Pyrimidines					
1	*t*-Bu	4-HC≡CPh	94	236	8·8(2H,s,H—C=N), 8·4(2H,d,J = 7 Hz,Ar), 7·6(2H,d,J = 7 Hz,Ar), 3·2(1H,s,C≡CH), 1·4(9H,s,Me_3)
2	*i*-Pr	4-HC≡CPh	52–5	222	8·6(2H,s,H—C=N), 8·4(2H,d,J = 7 Hz,Ar), 7·6(2H,d,J = 7 Hz,Ar), 3·2(1H,s,C≡CH), 3·0(1H,m,CH), 1·3(6H,d,J = 7 Hz,Me_2)
3	*c*-Hex	4-HC≡CPh	90–2	262	8·6(2H,s,H—C=N), 8·4(2H,d,J = 7 Hz,Ar), 7·6(2H,d,J = 7 Hz,Ar), 3·2(1H,s,C≡CH), 2·6(1H,m,CH), 1·9(4H,m,*c*-Hex), 1·5(6H,m,*c*-Hex)
4	Ph	4-HC≡CPh	184–6	256	9·0(2H,s,H—C=N), 8·5(2H,d,J = 7 Hz,Ar), 7·6(2H,d,J = 7 Hz,Ar), 7·4(5H,m,Ar), 3·2(1H,s,C≡CH)
5	4-FPh	4-HC≡CPh	154–6	274	9·0(2H,s,H—C=N), 8·5(2H,d,J = 7 Hz,Ar), 7·6(4H,m,Ar), 7·2(2H,m,Ar), 3·2(1H,s,C≡CH)
6	*t*-Bu	4-BrPh	97–100	290	8·8(2H,s,H—C=N), 8·3(2H,d,J = 7 Hz,Ar), 7·6(2H,d,J = 7 Hz,Ar), 1·4(9H,s,Me_3)
7	*i*-Pr	4-BrPh	60–3	276	8·7(2H,s,H—C=N), 8·3(2H,d,J = 7 Hz,Ar), 7·6(2H,d,J = 7 Hz,Ar), 3·0(1H,m,CH), 1·3(6H,m,Me_2)
8	*c*-Pr	4-BrPh	134	274	8·5(2H,s,H—C=N), 8·3(2H,d,J = 7 Hz,Ar), 7·6(2H,d,J = 7 Hz,Ar), 1·9(1H,m), 1.1(2H,m), 0.8(2H,m)
9	*c*-Hex	4-BrPh	108–110	316	8·8(2H,s,H—C=N), 8·3(2H,d,J = 7 Hz,Ar), 7·6(2H,d,J = 7 Hz,Ar), 2·5(1H,m,CH), 1·9(4H,m,*c*-Hex) 1·5(6H,m,*c*-Hex)
10	Me	4-BrPh	163–5	248	8·6(2H,s,H—C=N), 8·3(2H,d,J = 7 Hz,Ar), 7·6(2H,d,J = 7 Hz,Ar), 2·5(3H,s,Me)
11	Ph	4-BrPh	176–8	310	9·0(2H,s,H—C=N), 8·4(2H,d,J = 7 Hz,Ar), 7·6(3H,m,Ar), 7·5(4H,m,Ar)
12	3-FPh	4-BrPh	162–3	328	9·0(2H,s,H—C=N), 8·4(2H,d,J = 7 Hz,Ar), 7·6(4H,m,Ar), 7·2(2H,m,Ar)
13	4-FPh	4-BrPh	170–3	328	9·0(2H,s,H—C=N), 8·4(2H,d,J = 7 Hz,Ar), 7·6(4H,m,Ar), 7·2(2H,m,Ar)
4H-1,3-Thiazine					
14	t-Bu	4-HC≡CPh	Yellow oil	255	7·9(2H,d,J = 7 Hz,Ar), 7·6(2H,d,J = 7 Hz,Ar), 7·3(1H,s,C = H), 4·1(2H,s,CH_2), 3·2(1H,s,C≡CH), 1·2(9H,s,Me_3)
15	*t*-Bu	4-BrPh	192–4	309	8·0(2H,d,J = 7 Hz,Ar), 7·6(2H,d,J = 7 Hz,Ar), 7·1(1H,s,C=CH), 4·1(2H,m,CH_2), 1·2(9H,s,Me_3)
6H-1,3-Thiazine					
16	*t*-Bu	4-BrPh	Yellow oil	309	7·8(2H,d,J = 7 Hz,Ar), 7·5(2H,d,J = 7 Hz,Ar), 7·2(1H,s,C=CH), 4·2(2H,s,CH_2), 1·2(9H,s,Me_3)
6-Oxo-1,3-thiazine					
17	*i*-Pr	4-HC≡CPh	88–90	255	8·1(1H,s,C=CH), 8·0(2H,d,J = 7 Hz,Ar), 7·6(2H,d,J = 7 Hz,Ar), 7·2(1H,s,C=CH), 3·2(1H,m,CH), 1·2(6H,d,J = 7 Hz,Me_2)
18	*t*-Bu	4-BrPh	122–4	323	8·3(1H,s,C=CH), 7·8(2H,d,J = 7 Hz,Ar), 7·6(2H,d,J = 7 Hz,Ar), 1·4(9H,s,Me_3)
19	*i*-Pr	4-BrPh	100–103	309	8·1(1H,s,C=CH), 7·8(2H,d,J = 7 Hz,Ar), 7·6(2H,d,J = 7 Hz,Ar), 3·1(1H,m,CH), 1·2(6H,d,J = 7 Hz,Me_2)
4,6-Dioxo-1,3-thiazine					
20	*i*-Pr	4-BrPh	217	325	7·8(2H,d,J = 7 Hz,Ar), 7·6(2H,d,J = 7 Hz,Ar), 3·5(1H,m,CH), 1·6(1H,br,OH), 1·3(6H,d,J = 7 Hz,Me_2)
1,3-Dioxanes					
21	*t*-Bu	4-HC≡CPh, H_a	107–109	244	7·5(4H,m,Ar), 4·3(2H,m,CH_e), 3·8(2H,dd,J = 9,9 Hz,CH_a), 3·1(1H,s,C≡CH), 1·9(1H,m,CH), 0·9(9H,s,Me_3)
22	*t*-Bu	4-HC≡CPh, Me_e	Colorless oil	258	7·5(2H,d,J = 7 Hz,Ar), 7·3(2H,d,J = 7 Hz,Ar), 3·9(2H,dd,J = 3,9 Hz,CH_e), 3·5(2H,dd,J = 9,9 Hz,CH_a), 3·1(1H,s,C≡CH), 1·8(1H,m,CH), 1·5(3H,s,Me), 0·8(9H,s,Me_3)
23	*t*-Bu	4-BrPh, Me_e	77–80	312	7·5(2H,d,J = 7 Hz,Ar), 7·2(2H,d,J = 7 Hz,Ar), 3·8(2H,m,CH_e), 3·5(2H,m,CH_a), 1·8(1H,m,CH), 1·4(3H,s,Me), 0·7(9H,s,Me_3)
1,3-Dithianes					
24	*t*-Bu	4-HC≡CPh, H_a	149	276	7·4(4H,m,Ar), 5·1(1H,s,2-H), 3·1(1H,s,C≡CH), 2·9(2H,m,CH_e), 2.8(2H,m,CH_a), 1·7(1H,m,5H), 0·9(9H,s,Me_3)
25	*t*-Bu	4-BrPh, H_a	173	330	7·4(4H,m,Ar), 5·1(1H,s,2-H), 2·9(2H,m,CH_e), 2·8(2H,m,CH_a), 1·7(1H,m,5-H), 1·0(9H,m,Me_3)

[a] Abbreviations for substituents are Me methyl, *i*-Pr isopropyl, *t*-Bu *tert*-butyl, *c*-Pr cyclopropyl, *c*-Hex cyclohexyl, Ph phenyl, *e* equatorial, and *a* axial.

[^{1}H]NMR: δ 3·6 (2H,m,CH_2O), 3·2 (2H,m,CH_2N), 2·2 (3H,br,OH,NH_2), 1·3 (1H,m,CH), 0·8 (9H,s,$(CH_3)_3$).

2.3.2 3,3-Dimethyl-2-hydroxymethylbutyl 4-bromobenzamide (E)

A solution of 4-bromobenzoyl chloride (0·86 g) in diethyl ether (10 ml) was rapidly added to a vigorously stirred mixture of **D** (0·5 g) and sodium hydroxide (0·16 g) in water (10 ml) and diethyl ether (5 ml) at 0°C. Stirring was continued for 30 min then methylene chloride (30 ml) was added. The mixture was separated and the organic phase washed with dilute hydrochloric acid (20 ml), dried (sodium sulfate) and evaporated. The residue was chromatographed on a flash silica column eluting with ethyl acetate + hexane (1 + 1 by volume). The product (0·5 g) was a solid (m.p. 110–112°C). [^{1}H]NMR: δ 7·6 (4H,m,Ar), 7·0 (1H,br,NH), 3·6 (2H,m,CH_2O), 3·4 (2H,m,CH_2N), 1·5 (1H,m,CH), 1·0 (9H,s,$(CH_3)_3$).

2.3.3 2,2-Dimethyl-2-formylbutyl 4-bromobenzamide (F)

A solution of oxalyl chloride (0·17 ml) in dry methylene chloride (15 ml) was cooled to −70°C and stirred under nitrogen while a solution of dimethyl sulfoxide (DMSO; 0·28 ml) in methylene chloride (1 ml) was added slowly. After stirring for 15 min, a solution of **E** (0·51 g) in methylene chloride (25 ml) was added. Stirring was continued for 1 h when the solution was treated with triethylamine (1·2 ml) and allowed to warm to room temperature over 30 min. Dilute aqueous hydrochloric acid was added and the organic phase separated, washed with water, dried (sodium sulphate), and evaporated. The product (0·51 g) was obtained as a white solid (m.p. 120–123°C). [^{1}H]NMR: δ 9·9 (1H,s,CHO), 7·3 (4H,m,Ar), 6·5 (1H,br,NH), 3·4 (2H,m,CH_2), 2·5 (1H,m,CH), 1·0 (9H,s,$(CH_3)_3$).

*2.3.4 2-(4-Bromophenyl)-5-*tert*-butyl-4H-thiazine (15) and 5-*tert*-butyl-2-(4-ethynylphenyl)-4H-thiazine (14)*

A mixture of aldehyde **F** (0·5 g) and Lawesson's Reagent (0·65 g) in xylene (3 ml) was heated at reflux for 6 h. The cooled mixture was treated with aqueous sodium hydrogen carbonate solution and diethyl ether. After separation the ethereal solution was washed with brine, dried (sodium sulfate) and evaporated. Flash column chromatography eluting with hexane + ethyl acetate (3 + 1 by volume) yielded the desired product as a white solid (90 mg). The palladium coupling conditions described earlier with trimethylsilylacetylene were used to convert **15** to **14**. [^{13}C]NMR: δ for **15** 145·3, 132·5, 132·4, 131·8, 130·3, 126·6, 111·6 (=CH), 53·6 (CH_2), 35·1 ($C(CH_3)_3$), 29·5 (CH_3).

*2.3.5 2-(4-Bromophenyl)-5-*tert*-butyl-6H-thiazine (16)*

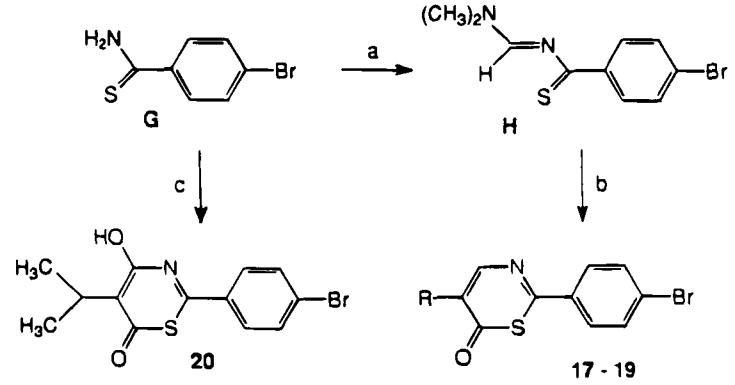
A mixture of **F** (2·5 g) and P_2S_5 (1·8 g) in xylene (10 ml) was heated at reflux for 3 h. After cooling, aqueous sodium hydrogen carbonate and diethyl ether were added and the mixture stirred overnight. The organic phase was washed with brine, dried (sodium sulfate) and evaporated. Preparative TLC on silica, developing with hexane + ethyl acetate (9 + 1 by volume), yielded the product as a viscous yellow oil (0·4 g). [^{13}C]NMR: δ 145·3, 132·5, 132·4, 131·8, 130·3, 126·6, 111·6 (=CH), 53·6 (CH_2), 36·8 ($C(CH_3)_3$), 29·5 (CH_3).

2.4 6-Oxo- and 4,6-Dioxo-1,3-thiazines (17–20) (Fig. 4) (Table 1)

The general routes used to prepare the 6-oxo- and, 4,6-dioxo-1,3-thiazines have already been described.[17] Thioacylformamidine **H**,[18] derived from 4-bromophenylthioamide (**G**),[19] was reacted with the appropriate alkylacetyl chloride in methylene chloride and triethylamine to give compounds **18** and **19** with further conversion of **19** to **17** as described above. Treatment of thioamide **G** with isopropylmalonic acid and $POCl_3$ gave 2-(4-bromophenyl)-5,6-dihydro-4*H*-5-isopropyl-1,3-thiazine-4,6-dione (**20**).[20]

2.5 1,3-Dioxanes and 1,3-Dithianes (Table 1)

Dioxanes **21**–**23** and dithianes **24** and **25** were synthesized by published procedures involving condensation



a: $(CH_3)_2NCH(OC_2H_5)_2$, CH_2Cl_2; b: RCH_2COCl, $(C_2H_5)_3N$, CH_2Cl_2; c: $(CH_3)_3CHCH(CO_2H)_2$, $POCl_3$

Fig. 4. Synthesis of 5-alkyl-2-aryl-6-oxo- or 4,6-dioxo-1,3-thiazines.

of 3,3-dimethyl-2-hydroxymethylbutan-1-ol or the corresponding dithiol, respectively, with 4-bromo- or 4-ethynylbenzaldehyde or 4-bromoacetophenone under acidic conditions to yield the desired compounds.[4,5,7]

3 BIOLOGY

Adult female house flies (*Musca domestica* L., SCR susceptible strain, 4–10 days after emergence, *c.*20 mg each) were treated topically on the ventrum of the abdomen with a candidate insecticide dissolved in acetone (0·5 μl). In tests with the synergist, piperonyl butoxide (PB) was applied topically to each fly at 5 μg in 0·5 μl acetone 2 h prior to the insecticide. Mortality data at 24 h were plotted on log-probit paper. Each experiment was repeated on at least three separate days with 10 or 20 flies per group and a dose differential of two-fold. LD_{50} values were generally reproducible within 1·5-fold between experiments.

The potency of compounds in blocking the GABA-gated chloride channel was assayed as inhibition of [^{3}H]EBOB binding with house fly head[9] and mouse brain membranes.[21] The house fly assay consisted of membranes sedimented at 50 000 *g* but not at 10 000*g* (100 μg protein)[22] in phosphate buffer (50 mM, pH 7·4, 1·0 ml) containing [^{3}H]EBOB (final concentration 1 nM) with incubation for 1 h at 22°C. Mouse brain membranes (50 μg protein) were prepared and assayed by the same procedure except the incubation was for 1 h at 37°C. Candidate inhibitors were added at the same time as the radioligand for direct competition studies. Incubation mixtures were filtered on Whatman GFC glass fiber filters and washed with the phosphate buffer (3 × 5 ml) at 5°C. Specific binding was considered to be the difference between total binding (1 nM [^{3}H]EBOB) and non-specific binding (1 nM [^{3}H]EBOB plus 5 μM unlabeled EBOB). Each experiment was repeated three or more times with duplicate assays. The house fly and mouse receptor assays were made with the same inhibitor solutions in the same experiments and are therefore directly comparable.

4 RESULTS

4.1 Structure–activity relationships (Table 2)

4.1.1 Pyrimidines (1–13)

Pyrimidines **1–3** are potent chloride channel blockers and toxicants for house flies. The ethynylphenyl analogs (**1–5**) are always much more effective than the analogous bromophenyl compounds (**6**, **7**, **9**, **11** and **13**). The activity decreased for the 5-substituent in the general order *tert*-butyl > isopropyl ≈ cyclohexyl ≈ cyclopropyl > methyl, phenyl or fluorophenyl.

4.1.2 1,3-Thiazines (14–16)

In this series the only quite effective compound was 5-*tert*-butyl-2-(4-ethynylphenyl)-4*H*-1,3-thiazine (**14**) while the analogous bromophenyl-4*H*-1,3-thiazine (**15**) and -6*H*-1,3-thiazine (**16**) were almost inactive.

4.1.3 6-Oxo- and 4,6-dioxo-1,3-thiazines (17–20)

Three 6-oxo-1,3-thiazines (**17–19**) including one with a 4-ethynylphenyl moiety were inactive in all of the assays. This was also true for 2-(4-bromophenyl)-5-isopropyl-4,6-dioxo-1,3-thiazine (**20**).

4.1.4 1,3-Dioxanes and 1,3-dithianes (21–25)

5-*tert*-Butyl-2-(4-ethynylphenyl)-1,3-dioxane in the *trans* configuration (**21**) has high activity[5] but 5-*tert*-butyl-2-(4-ethynylphenyl)-2-methyl-1,3-dioxane as the *cis* isomer (**22**) had low activity, as did its bromo analog (**23**). Ethynylphenyl *trans*-dithiane **24** is of similar activity to the analogous *trans*-dioxane (**21**) and they are more effective than the bromophenyl *trans*-dithiane (**25**).

4.2 Mode of action

Two observations indicate that the new pyrimidines and thiazines probably act as chloride channel blockers. First, the poisoning signs are identical to those described for EBOB in both mice[1] and house flies.[23] Second, the potency of the pyrimidines, thiazines and dioxanes as inhibitors of [^{3}H]EBOB binding in the house fly head membranes is a fair predictor of their topical toxicity to house flies with PB (Fig. 5). Thus, 10 compounds active in the house fly receptor assay are all effective as insecticides with increasing receptor potency

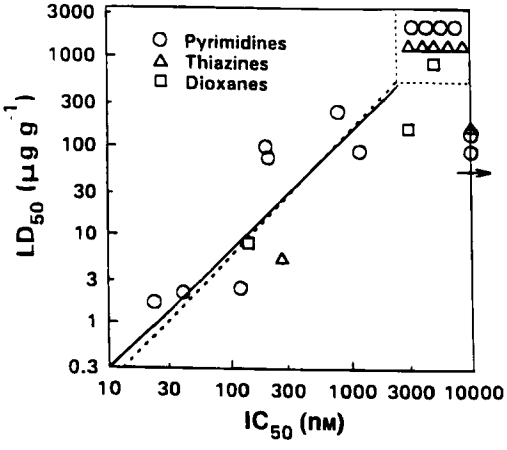


Fig. 5. Correlation for pyrimidines, thiazines and dioxanes between potency as inhibitors of [^{3}H]EBOB binding to house fly head membranes and as toxicants to house flies (pretreated with PB). Inactive compounds (IC_{50} > 10 000 nM and topical LD_{50} > 250 μg g^{-1}) are shown in the upper right box. A correlation coefficient of 0·78 is obtained by linear regression for the 10 compounds with discrete IC_{50} and LD_{50} values (Table 2). The dotted line is taken directly from the data of Deng *et al.*[9] for comparable experiments with polychlorocycloalkanes, trioxabicyclooctanes, dithianes, silatranes and picrotoxinin to show how closely they fit the same relationship.

TABLE 2
Biological Activities of Aryl Heterocyclic Compounds

	R-heterocycle-R'		*Chloride channel* IC_{50} (*nM*)[b]		*House fly* LD_{50} ($\mu g\ g^{-1}$)[c]	
No.	*R at C-5*[a]	*R' at C-2*[a]	*House fly*	*Mouse*	*−PB*	*+PB*
Pyrimidines						
1	*t*-Bu	4-HC≡CPh	23	21	5·5	1·7
2	*i*-Pr	4-HC≡CPh	40	290	8·5	2·2
3	*c*-Hex	4-HC≡CPh	120	21	11	2·5
4	Ph	4-HC≡CPh	200	1100	>250	100
5	4-FPh	4-HC≡CPh	>10000	2900	>250	140
6	*t*-Bu	4-BrPh	210	2100	110	75
7	*i*-Pr	4-BrPh	>10000	4000	125	90
8	*c*-Pr	4-BrPh	800	>10000	300	250
9	*c*-Hex	4-BrPh	1200	440	350	90
10	Me	4-BrPh	>10000	>10000	>250	>250
11	Ph	4-BrPh	>10000	>10000	>250	>250
12	3-FPh	4-BrPh	>10000	>10000	>250	>250
13	4-FPh	4-BrPh	>10000	>10000	>250	>250
4H-1,3-Thiazines						
14	*t*-Bu	4-HC≡CPh	270	170	27	5·5
15	*t*-Bu	4-BrPh	>10000	830	>250	>250
6H-1,3-Thiazines						
16	*t*-Bu	4-BrPh	>10000	>10000	>250	170
6-Oxo-1,3-thiazine						
17	*i*-Pr	4-HC≡CPh	>10000	>10000	>250	>250
18	*t*-Bu	4-BrPh	>10000	>10000	>250	>250
19	*i*-Pr	4-BrPh	>10000	>10000	>250	>250
4,6-Dioxo-1,3-thiazine						
20	*i*-Pr	4-BrPh	>10000	>10000	>250	>250
1,3-Dioxanes						
21	*t*-Bu	4-HC≡CPh, H_a	140	25	2·6	0·80
22	*t*-Bu	4-HC≡CPh, Me_e	3000	>10000	>250	160
23	*t*-Bu	4-BrPh, Me_e	>10000	>10000	>250	>250
1,3-Dithianes						
24	*t*-Bu	4-HC≡CPh, H_a	61	27	1·4	0·24
25	*t*-Bu	4-BrPh, H_a	300	1200	5·0	1·3

[a] For abbreviations see Table 1.
[b] 50% inhibition of [^{3}H]EBOB binding.
[c] Topical LD_{50} with test compound alone and 2 h after treatment with PB at 250 $\mu g\ g^{-1}$. Acetone carrier solvent, except **10** and **11** applied in THF. >250 designates no mortality at this dose.

related to house fly toxicity ($r = 0·78$) whereas 13 compounds inactive in the [^{3}H]EBOB assay had little or no insecticidal activity. The correlation coefficient for data with the mouse brain receptor versus toxicity to house flies (with PB) is 0·90 for the 10 compounds with discrete IC_{50} and LD_{50} values (correlation figure not shown).

5 DISCUSSION

Discoveries during the 1980s and 1990s led to the recognition that many types of insecticide act by disrupting the GABA-gated chloride channel.[24] The finding that trioxabicyclooctanes with suitable 1- and 4-substituents were very active[1,3] prompted further investigations, including candidate replacements for the central moiety, such as the dioxane and the much more active dithiane ring systems.[4] It is possible that the dithianes are effective by positioning the 2- and 5-substituents in a manner mimicking the equivalent 1- and 4-groups of the trioxabicyclooctanes. The corresponding 2-(4-halophenyl)-dioxanes were of very low activity.[4,5] It was therefore surprising to find that *trans*-5-*tert*-butyl-2-(4-ethynylphenyl)-dithiane (**24**) and

-dioxane (**21**) fall in the same potency range,[5] i.e. sulfurs and oxygens in these series are both effective in conferring activity.

The dithianes and dioxanes were examined as individual *cis*- and *trans*-isomers. The 2,5-substituent orientation of the *trans* form in each case approaches that of the 1,4-substituents of the trioxabicyclooctanes. This cannot be achieved in the case of *cis*-dithianes and -dioxanes, unless a twist-boat conformation is invoked at the site of action.[4] The *cis*- and *trans*-dithianes are active with H or methyl at C-2[2,4] whereas the *cis*-dioxanes with methyl at C-2 (compounds **22** and **23**) are of very low potency. It is possible that, under physiological conditions, the ring may open and reform, allowing isomer interconversion. In such a case the *cis*-dioxanes with methyl at C-2 would be expected to hinder any ring-opening mechanism and the parent molecule would remain in the less active *cis* form.

The present study examines candidate pyrimidine and thiazine replacements for the trioxabicyclooctane, dithiane and dioxane ring systems. In the 5-alkyl-2-arylpyrimidines and -thiazines and their analogs the heterocyclic spacer unit maintains the *tert*-butyl and ethynylphenyl or equivalent substituents in a linear or planar relationship. The most effective pyrimidines (**1–3**) and thiazine (**14**) are less active than the analogous dioxanes and dithianes (this study)[5] and trioxabicyclooctanes.[25] The free electrons of the heterocyclic oxygens and sulfurs may enhance binding[8] by supplying suitable steric and electrostatic fields whereas the nitrogen lone pair electrons of the pyrimidines, thiazines, oxothiazines and dioxothiazines are delocalized making them less available for use at the binding site. The shorter bond length of nitrogen versus oxygen and sulfur may also be important.

Optimal substituents at the 5-position of the pyrimidines are generally *tert*-butyl > isopropyl or cyclohexyl or cyclopropyl > aryl, and at the 2-position are ethynylphenyl > bromophenyl (this study) which follows the pattern originally recognized with the analogous 1- and 4-substituents of the prototype trioxabicyclooctanes.[1,3,25] With the dithianes the 5-cyclohexyl and 5-aryl groups render the compounds inactive.[4] The linearity of the 1- and 4-substituents of the trioxabicyclooctanes cannot be achieved in the dithiane series which holds the 2- and 5-substituents in a parallel relationship which may cause a steric limitation at the 5-position. In the pyrimidines, 5-cyclohexyl confers high potency indicating that the problem with phenyl or fluorophenyl is not steric but probably electronic caused by further delocalization of the heterocyclic ring electrons leading to loss of activity.

The GABA-gated chloride channel blockers are often toxic to mammals as well as insects. The LD_{50} values of pyrimidines **1**, **2** and **3** and dioxane **21** for mice treated intraperitoneally[1] are 0·6, 1·5, 1·0 and 15 mg kg^{-1} (results reproducible within 1·5-fold) compared with topical LD_{50} values for house flies (averaged with and without PB) of 3·6, 5·4, 6·7 and 1·7 $\mu g\ g^{-1}$, respectively, establishing unfavorable selectivity for the pyrimidines relative to the dioxane examined. Although some compounds in the present study are more potent inhibitors with the house fly receptor and others with the mouse preparation, there are no clear patterns correlated with selective toxicity.

The sequential modifications of the heterocyclic spacer group from trioxabicyclooctane to dioxane, dithiane, pyrimidine and thiazine (Fig. 1) are not expected to change the toxicologically relevant target, an hypothesis that can be tested by comparing the potency of the compounds in the [^{3}H]EBOB binding assay with their toxicity to house flies[9] (Fig. 5). PB is used as a synergist to minimize cytochrome P450-dependent oxidative detoxification. The correlation coefficient obtained for the 10 active pyrimidines, thiazine and dioxanes in this study ($r = 0·78$), and the fact that the 13 inactive molecules at the house fly receptor had little or no insecticidal activity at the concentrations tested, indicate that all of the compounds act at the same or closely coupled sites. The correlation line obtained for the pyrimidines, thiazine and dioxanes in the present study almost coincides with the corresponding line obtained earlier for picrotoxinin and selected trioxabicyclooctanes, dithianes, silatranes and polychlorocycloalkanes.[9] The present study therefore expands the list of putative chloride channel blockers active as insecticides so that, in addition to the compounds mentioned above and the aryl pyrazoles known before,[9,23,24,26] it now also includes certain pyrimidines, thiazines and dioxanes.

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